

## CLAIMS

1. A DNA expression cassette comprising:
  - (a) a partially randomized nucleic acid sequence having a 5' and a 3' end and including a first segment and a second segment,
    - the first segment comprising a series of 0 to 23 bases linked 3' to a plurality of at least four consecutive adenylyl residues linked 3' to a randomized nucleic acid sequence of from 11 to 27 bases long;
    - the second segment consisting of a sequence complementary to the first segment sequence and being linked to the 3' end of the first segment through its 5' end by a polymerase primer hairpin linker having the sequence  $N^1_nN^2_mN^3_n$ , where  $N^3$  is complementary to  $N^1$ ;
    - $n$  is a number greater than or equal to 2; and
    - $m$  is a number from 1 to 40;
  - and
  - (b) a pol III promoter having a TATA box, operably linked to the partially randomized nucleic acid sequence, the pol III promoter being modified to allow transcription from the promoter to begin at the first base of the randomized nucleic acid sequence.
2. The DNA expression cassette of claim 1, wherein the series of from 0 to 23 bases is at least one base and comprises a restriction site.
3. The DNA expression cassette of claim 1, wherein the pol III promoter is selected from the group consisting of H1 RNA promoters, U6 snRNA promoters, promoters for tRNA genes, and promoters for the adenovirus VA genes.
4. The DNA expression cassette of claim 1, wherein the pol III promoter is inducible.
5. The DNA expression cassette of claim 1, further comprising a viral particle for packaging a nucleic acid comprising the expression cassette.
6. The DNA expression cassette of claim 4, further comprising an inducible operator sequence 5' to the TATA box.

7. The DNA expression cassette of claim 6, wherein the inducible operator sequence is the tetO operator.
8. The DNA expression cassette of claim 1, wherein the first two nucleotides of N<sup>2</sup> are a sequence of two thymidyl residues.
9. A library of DNA expression cassettes, each expression cassette comprising:
  - (a) a partially randomized nucleic acid sequence having a 5' and a 3' end and including a first segment and a second segment,
    - the first segment comprising a series of 0 to 23 bases linked 3' to a plurality of at least four consecutive adenylyl residues linked 3' to a randomized nucleic acid sequence of from 11 to 27 bases long;
    - the second segment consisting of a sequence complementary to the first segment sequence and being linked to the 3' end of the first segment through its 5' end by a polymerase primer hairpin linker having the sequence N<sup>1</sup><sub>n</sub>N<sup>2</sup><sub>m</sub>N<sup>3</sup><sub>n</sub>, where N<sup>3</sup> is complementary to N<sup>1</sup>;
    - n is a number greater than or equal to 2; and
    - m is a number from 1 to 40;
  - and
  - (b) a pol III promoter having a TATA box, operably linked to the partially randomized nucleic acid sequence, the pol III promoter being modified to allow transcription from the promoter to begin at the first base of the randomized nucleic acid sequence.
10. The library of claim 9, wherein the pol III promoter is inducible.
11. The library of claim 9, wherein each DNA expression cassette is packaged in a viral particle.
12. The library of claim 9, wherein each DNA expression cassette is included in a cell genome.
13. The library of claim 9, wherein each DNA expression cassette is self-replicating.

14. The library of claim 10, further comprising an inducible operator sequence 5' to the TATA box.

15. The library of claim 9, wherein the first two nucleotides of N<sup>2</sup> are a sequence of two thymidyl residues.

16. A method for producing a library of DNA expression cassettes comprising:

(a) synthesizing a plurality of partially randomized nucleic acid sequences, each having a 5' and a 3' end and comprising;

a series of 0 to 23 bases linked at its 3' end to

a plurality of at least four consecutive adenylyl residues linked at its 3' end to a randomized nucleic acid sequence of from 11 to 27 bases long linked at its 3' end to

a polymerase primer hairpin linker having the sequence N<sup>1</sup><sub>n</sub>N<sup>2</sup><sub>m</sub>N<sup>3</sup><sub>n</sub>, where

N<sup>3</sup> is complementary to N<sup>1</sup>;

n is a number greater than or equal to 2; and,

m is a number from 1 to 40

wherein the randomized nucleic acid sequence is different in each of the expression cassettes in the library;

(b) extending each partially randomized nucleic acid sequence from the polymerase primer hairpin linker using the nucleic acid sequence 5' to the polymerase primer hairpin linkers as a template;

(c) denaturing each of the extended partially randomized nucleic acid sequences;

(d) annealing a 5' primer to the 5' end and a 3' primer to the 3' end of each of the denatured partially randomized nucleic acid sequences;

(e) ligating each annealed partially randomized nucleic acid sequence into a separate expression vector comprising a modified pol III promoter having a TATA box, wherein the modified pol III promoter is operably linked to the extended partially randomized nucleic acid sequence, the pol III promoter being modified whereby transcription from the promoter begins at the first base of the randomized nucleic acid sequence; and,

(f) generating a complementary strand to each partially randomized nucleic acid sequence, thereby forming a complete DNA expression cassette.

17. The method of claim 16, wherein the series of 0 to 23 bases of the synthesizing step is at least one base and comprises a restriction site.

18. The method of claim 16, wherein the generating step further comprises transforming competent bacteria with the expression vectors containing the partially randomized nucleic acid sequences.

19. The method of claim 16, wherein the generating step comprises *in vitro* synthesis of a complementary strand to each partially randomized nucleic acid sequence using a DNA polymerase.

20. The method of claim 16, wherein the first two nucleotides of N<sup>2</sup> is a sequence of two thymidyl residues.

21. A kit comprising a library of siRNA expression cassettes enclosed in one or more containers, each expression cassette of the library comprising:

i. a partially randomized nucleic acid sequence having a 5' and a 3' end and including a first segment and a second segment,  
the first segment comprising a series of 0 to 23 bases linked 3' to a plurality of at least four consecutive adenylyl residues linked 3' to a randomized nucleic acid sequence of from 11 to 27 bases long;  
the second segment consisting of a sequence complementary to the first segment sequence and being linked to the 3' end of the first segment through its 5' end by a polymerase primer hairpin linker having the sequence N<sup>1</sup><sub>n</sub>N<sup>2</sup><sub>m</sub>N<sup>3</sup><sub>n</sub>, where N<sup>3</sup> is complementary to N<sup>1</sup>;  
n is a number greater than or equal to 2; and  
m is a number from 1 to 40;  
and

ii. a pol III promoter having a TATA box, operably linked to the partially randomized nucleic acid sequence, the pol III promoter being modified to allow transcription from the promoter to begin at the first base of the randomized nucleic acid sequence.

22. The kit of claim 21, further comprising vectors, primers and other reagents for constructing the library.